Emirates Journal of Food and Agriculture. 2023. 35(7): 674-682
doi: 10.9755/ejfa.2023.v35.i7.3107
http://www.ejfa.me/

RESEARCH ARTICLE

Antioxidant and inhibitory activities of α-Amylase, α-Glucosidase and Angiotensin-I of protein hydrolysates from "Sac-Beh" quality protein maize (QPM)

Nidia Isabel Jiménez Suaste[1], Luis Chel-Guerrero[2], Roberto Zamora Bustillos[3], David Betancur- Ancona[2]*

[1]Posgrado Institucional de Ciencias Agropecuarias y Manejo de Recursos Naturales y Tropicales, UADY. Carretera Mérida-Xmatkuil km 15.5, Mérida, Yucatán, México CP. 97315, [2]Facultad de Ingeniería Química, Campus de Ciencias Exactas e Ingenierías, Universidad Autónoma de Yucatán. Periférico Nte. Km. 33.5, Tablaje Catastral 13615, Col. Chuburná de Hidalgo Inn, 97203. Mérida, Yucatán, México, [3]Avenida Tecnológico s/n Conkal, Yucatán. Conkal, Yucatán. C.P. 97345. Contacto.

ABSTRACT

Diabetes and hypertension are health problems with high mortality rates. Protein hydrolysates with antidiabetic and antihypertensive potential are suitable for making functional foods that could lower blood glucose and blood pressure, improve insulin absorption, and inhibit the enzymes involved in the development of these diseases. The protein hydrolysis was realized using Alcalase®, Flavourzyme®, and the sequential system Alcalase®-Flavourzyme®. The inhibitory activity, antioxidant activity, and ACE-1, α-Amylase, α-Glucosidase enzymes of protein hydrolysates derived from QPM Sac-Beh *Zea mays* L. were evaluated by *in vitro* assays. The hydrolysates with the highest degree of hydrolysis (90 min) were used to evaluate bioactivity. The maximum values for antioxidant assays were 9.1 Trolox equivalents/mg protein for Alcalase®-Flavourzyme®, 45.51% decolorization of 2,2-diphenyl-1-picrylhydrazyl (DPPH); the inhibition of α-Amylase with the Alcalase® system was 45.84%, for α-Glucosidase was 64.3% for the Flavourzyme® system; the IC_{50} value for the mean concentration of ACE-I enzyme activity with the Alcalase®-Flavourzyme® system was 73.09µg/ml with a percentage inhibition of 69.06%. Hydrolysates from the Sac-Beh variety of quality protein maize (QPM) could be used in the elaboration of pharmaceutical or functional foods that work as adjuvants in the treatment of people with hypertension, type II diabetes mellitus, as well as antioxidants.

Keywords: Maize; Diabetes; Hypertension; Protein hydrolysates; Sac-Beh; Angiotensin-I

INTRODUCTION

In the last decade, the growth and acceptance of protein hydrolysates have been substantial. This significant increase was propelled by the growing rates of metabolic and cardiovascular diseases and by the technological advances in the production and synthesis of peptides which has led to an exponential increase in the research for bioactive hydrolysates (Pasarin and Rovinaru, 2023).

Hydrolysis with commercial proteolytic enzymes is one of the most efficient processes for producing bioactive peptides (Chalamaiah et al., 2018). Thus, vegetal resources have attracted attention as an alternative source of bioactive compounds due to their low cost and good nutritional quality (Esfandi et al., 2019; Aldhanhani et al., 2022a). They may exhibit antioxidant, antihypertensive (Chuesiang and Sanguandeekul, 2015; Al Shaibani et al., 2022), immunomodulatory, antithrombotic, anticancer, and antihyperglycemic, among other types of activities (Shahidi and Ambigaipalan, 2015; Aldhanhani et al., 2022b). Corn gluten meal was hydrolyzed with Alcalase®, Flavourzyme®, Alcalase® + Flavourzyme® and Flavourzyme® + Alcalase®. At the substrate concentration of 10%, corn protein hydrolysate catalyzed by Alcalase had a degree of hydrolysis of 17.83%, which was higher than that by Flavourzyme® (3.65%). The hydrolysate catalyzed by Alcalase® + Flavourzyme® exhibited better antioxidant activities and was further purified (Du-xin et al, 2016).

***Corresponding author:**
David Betancur- Ancona, Facultad de Ingeniería Química, Campus de Ciencias Exactas e Ingenierías, Universidad Autónoma de Yucatán. Periférico Nte. Km. 33.5, Tablaje Catastral 13615, Col. Chuburná de Hidalgo Inn, 97203. Mérida, Yucatán, México.
Phone: +52 (999) 9460956. **E-mail:** bancona@correo.uady.mx

Received: 03 October 2022; **Accepted:** 20 June 2023

Several studies have reported the production of functional products by the incorporation of protein hydrolysates. Karimi et al, (2021) reported the fortification of bread with maize germ protein hydrolysate for enhancement of *in vitro* antioxidant potential of the bread before and after digestion and α-amylase inhibition during digestion. Similarly, Fitzgerald et al. (2014) reported that the addition of *Palmaria palmata* protein hydrolysate to bread enhanced the inhibitory activity angiotensin converting enzyme.

Maize as a natural resource is the most important cereal in terms of production volume worldwide, it is a commodity in the international stock market and Mexico is the eighth largest maize-producing country in the world. In Mexico, genetic improvement programs have been developed through the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP) to generate QPM-type native maize varieties. A new alternative is the modification of *Zea mays* L. maize with the gene called opaque-2, which increases the production of lysine and tryptophan in the grain, improving the protein quality of this food, hence being called Quality Protein Maize (QPM) (Vasal et al., 1993). Maize opaque-2 possesses a recessive mutant gene (o2) that increases the content of the other protein fractions. As a result, a protein matrix with different amino acid distribution and a doubling of Lys and Trp content is generated. Thus, the expression of this gene makes the nutritional value of this maize superior to normal maize (Hasjim et al., 2009). In the Yucatán Peninsula, there is the Sac-Beh maize variety (Mayan name meaning white road). This variety has its own characteristics such as resistance to pests and water stress, good development during sowing, relevant physicochemical properties, and an appreciated nutritional value. The objective was the evaluation of bioactive effects of the protein hydrolysates obtained from the QPM Sac-Beh variety. The antioxidant, antihypertensive, and inhibitory activities of α-Amylase, and α-Glucosidase enzymes of these protein hydrolysates obtained by enzymatic hydrolysis through *in vitro* methods were evaluated.

MATERIALS AND METHODS

Raw materials

Maize grains *(Zea mays* L.) native variety QPM Sac-Beh, were provided by the INIFAP located in the municipality of Mocochá, in the state of Yucatán, México. They were transferred to the Food Science laboratory of the Universidad Autónoma de Yucatán where they were stored at -10°C until use.

Flour extraction

The grains were manually sorted to remove impurities and damaged or broken kernels. Sac-Beh *Zea mays* L. grains were milled in a Pulvex mill until a flour capable of passing through 20 mesh (0.85 mm) was obtained, then the flour was milled again in Cyclotec 1093 mills (Tecator, Sweden) until a flour capable of passing through 60 mesh (0.24 mm) was obtained. Moisture (AOAC 930.0) and crude protein (AOAC 978.04) determination of QPM Sac-Beh flour was conducted following the official methods of the Association of Official Agricultural Chemists International (AOAC, 2012).

Protein concentrate extraction from QPM (Zea mays L.)

The protein concentrates of Sac-Beh Zea mays L maize were obtained according to the method indicated by Wang et al. (2001) with a slight modification that consisted of changing the isoelectric point to pH 4, removing the supernatant, and drying by freeze- drying.

Enzymatic hydrolysis of QPM protein concentrates

Hydrolysis was done under controlled conditions of temperature, pH, and agitation in a 1000 ml reaction vessel equipped with an agitator, thermometer, and Ohaus model ST20 potentiometer (Fig. 1). The protein concentrate was suspended in distilled water to produce a 5% (w/v) protein solution.

Enzymatic hydrolysis was performed using 3 treatments; in the first two, the commercial enzymes Alcalase® (P4868 Sigma-Aldrich) and Flavourzyme® (P6110 Sigma-Aldrich) were used individually. A 5% w/v suspension was prepared in relation to the protein content present in the concentrate, and the enzyme-substrate ratio was 1:10 %. The pH was adjusted to 7.5 with NaOH 1N, the Alcalase® enzyme (0.3 AU/ml) was added and the hydrolysis reaction was performed at 0, 15, 30, 30, 45, 60, and 90 min, at a temperature of 50 °C with constant agitation. To complete the hydrolysis, the enzyme was inactivated by the addition of HCl 1N until the pH was adjusted to 5.0. In the case of

Fig 1. Reaction system for enzymatic hydrolysis of Sac-Beh maize (QPM) protein concentrate.

the Flavourzyme ®, a 5% w/v suspension was prepared and the enzyme-substrate ratio was 1:10 %. The reaction was conducted at 50 °C, the pH was adjusted to 7 with NaOH 1N, Flavourzyme® enzyme (50 LAPU/g) was added and the hydrolysis reaction was performed at 0, 15, 30, 45, 60, and 90 min. Lastly, to complete the hydrolysis, the protease was inactivated by heating at 85°C for 20 min; in the third treatment, the Alcalase®-Flavourzyme® system was used sequentially at the same times above cited using half time for each enzyme.

A randomized complete block design was used, where the blocking factor was the different enzyme systems, the main factor evaluated was the hydrolysis time (0, 15, 45, 60, and 90 min), and the response variable was the degree of hydrolysis. Experiments were performed in triplicate.

Degree of hydrolysis

The degree of hydrolysis (DH) was determined by measuring the soluble nitrogen content in 10% trichloroacetic acid, following the methodology proposed by Margot et al. (1994). Subsequently, the hydrolysate with the highest degree of hydrolysis from each system (Alcalase®, Flavourzyme®, and Alcalase®-Flavourzyme®) was selected, and the protein content was determined in triplicate, using the method of Lowry et al., (1951).

Determination of antioxidant activity

DPPH assay

To determine the free radical scavenging capacity of the hydrolysates, the ones with the highest degree of hydrolysis, the ones obtained at a time of 90 min, were selected. The analysis consisted of using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Shimada et al., 1992). All the analyses were performed in triplicate.

ABTS assay

It was determined according to the method of Pukalskas et al. (2002). A stock solution of cation radical 2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was prepared by dissolving 54.8 mg of ABTS (2 mM) in 50 ml of phosphate-buffered saline (PBS 0.01 M, pH 7.4). The ABTS·+ cation radical was created by reacting 10 ml of the ABTS stock solution with 40 µL of 70 mM $K_2S_4O_8$ prepared 16 -17 hrs prior use. Measurement of the antioxidant activity of the samples was performed by mixing 10µL of hydrolysate and 990 µL of diluted ABTS radical in 2 ml microtubes and subsequently reading the absorbance at 734 nm after 6 min.

α-Amylase enzyme inhibition

In test tubes, 200 µl of α-Amylase 13 U/ml solution (Type VI-B from porcine pancreas, Sigma Aldrich, lot 27H0463, previously dissolved in 20 mM sodium phosphate buffer, pH 6.9) and 200 µl of the sample (previously selected protein hydrolysate with the highest degree of hydrolysis, dissolved in water at a concentration of 107 mg protein/ml) were added and incubated at 25 °C for 10 min. Subsequently, to start the reaction, 200 µl of a 1% starch solution (Maizena®) (previously dissolved in 20 mM sodium phosphate buffer, pH 6.9 and boiled for 15 min) was added to each tube and incubated at 25°C for another 10 min. When this time was over, 400 µl of DNS color reagent (previously prepared with 1% 3,5-Dinitrosalicylic acid, 30% Na-K tartrate in 0.4 M sodium hydroxide) was added to each tube and incubated in a water bath at 100°C for 5 min to stop the reaction. Finally, the tubes were left to cool at room temperature for 5 min, and 6 ml of water was added to each tube, then they were shaken by inversion of the tube and the absorbance was measured at 540 nm in a Jenway 7305 UV-VIS spectrophotometer, using a buffer solution photometric blank (Miller, 1959).

Inhibition tests were performed in triplicate. Inhibitory activity was calculated as the percentage inhibition of α-Amylase enzyme activity and was determined according to equation (1):

$$\%\alpha - \text{Amilase inhibition} = \left(\frac{(\text{ESUa} - \text{BESUa}) - (\text{ESUSAa} - \text{BESUSAa})}{(\text{ESUa} - \text{BESUa})} \right) \text{x}100 \quad (1)$$

Where: ESUa is the enzyme-substrate mixture absorbance; BESUa is the blank-enzyme-subtrate mixture absorbance; ESUSAa is the enzyme-substrate-sample mixture absorbance; and BESUSAa is the blank-enzyme-substrate-sample mixture absorbance.

α-Glucosidase enzyme inhibition

For the α-Glucosidase enzyme inhibition assay. 20 µl of a solution of α-Glucosidase 2U/ml (Type I from *Saccharomyces cerevisiae*, Sigma Aldrich, lot SLBP0778V, dissolved in 50 mM potassium phosphate buffer, pH 6.8) was placed in a 1.5 ml tube. 5 µl of the sample (previously selected protein hydrolysate with the highest degree of hydrolysis, dissolved in water at a concentration of 107 mg protein/ml) was added and incubated at 37°C for 20 min. Subsequently, 20 µl of p-nitrophenyl-α-D-glucopyranoside (PNPG, 1 mM) was added as substrate to initiate the reaction and incubated at 37°C for 20 min, after this time 50 µl of Na2CO3 solution (1 M) were added to terminate the reaction and each well was completed to a volume of 150 µl with buffer solution. The amount of released p-nitrophenol was measured at 405 nm using a Jenway 7305 UV/VIS spectrophotometer and buffer solution as photometric blank. For all tests, the inhibition assay was performed in quadruplicate. Acarbose 50mM, a drug approved as an

α-Glucosidase inhibitor that can inhibit both α-Amylase and α-Glucosidase (Mojica and González de Mejía, 2015) was used as a positive control. The equation (2) was used for the calculations.

$$\%\alpha - \text{Glucosidase inhibition} = \frac{(ESUa - BESUa) - (ESUSAa - BESUSAa)}{(ESUa - BESUa)} x100 \quad (2)$$

Where: ESUa is the enzyme-substrate mixture absorbance; BESUa is the blank-enzyme-subtrate mixture absorbance; ESUSAa is the enzyme-substrate-sample mixture absorbance; and BESUSAa is the blank-enzyme-substrate-sample mixture absorbance.

Angiotensin-I converting enzyme inhibitory activity

The method used was that of Hayakari et al., (1978). A stock solution of the protein hydrolysate sample was prepared and dilutions were made at concentrations of 0.8, 0.6, 0.4, 0.2, and 0.1 mg protein/ml. The samples were read with a UV spectrophotometer Vis EvolutionTM 300 (Thermo Fisher Scientific, USA) at 382 nm. To calculate the 50 % ACE-I inhibition concentration (IC50), the following was performed: for each hydrolysate concentration, the percentage of activity was calculated using the equation (3):

$$\%ACE - I \ \ inhibition = \left(\frac{A - C}{A - B}\right) x\ 100 \quad (3)$$

Where: A is the absorbance of the sample; B is the absorbance of the control; C is the absorbance of the blank.

For the hydrolysates from the Sac-Beh maize variety presenting the highest inhibitory activity, these obtained with the enzyme systems Alcalase®, Flavourzyme®, or Alcalase®-Flavourzyme®, the value of the IC_{50} was determined.

Statistical analysis

All results were analyzed by descriptive statistics using measures of central tendency (mean) and dispersion (standard deviation). The standard deviation of each protein concentrate hydrolysis and biological activities (antioxidant, enzyme inhibition, α-Amylase, α-Glucosidase, and Angiotensin-I) were evaluated by analysis of variance ANOVA for a two-way model where a factor was enzymatic system and other was reaction time and a comparison of means by Duncan's method to establish the differences between treatments. All these analyses were performed using the Statgraphics Plus Version 19 computational package.

RESULTS AND DISCUSSION

Enzymatic hydrolysis of protein concentrate

The protein concentrate was obtained from QPM with a protein content of 47% and a yield of 8%, proving to be a good option as raw material for enzymatic hydrolysis with endoprotease (Alcalase®), exopeptidase (Flavourzyme®), and the sequential system Alcalase®-Flavourzyme®.

The degree of hydrolysis for the hydrolysates with the Alcalase® enzyme ranged from 15-72.60%, and with the hydrolysates with the Alcalase®-Flavourzyme® system ranged from 17-79%. The results for the hydrolysates with the Flavourzyme® enzyme indicated a higher DH for QPM *Zea mays* L., ranging from 15.7%-83.7% (Fig. 2). This is explained given that Flavourzyme® is a mixture of endo and exopeptidase enzymes, so it can hydrolyze in more sites of the protein chain and can produce small size peptides and even free amino acids; on the other hand, Alcalase® is an endo-protease that cuts peptide bonds inside polypeptide chains, therefore quantitatively it has fewer possibilities to hydrolyze fewer sites of the chain and produces mainly small and medium size peptides (de Castro et al., 2017). Similar results have been reported for flaxseed and barley hydrolysates, where higher DH was achieved with Flavourzyme® compared to Alcalase® (Kulczyk et al., 2016). On the other hand, the highest DH value obtained from QPM Sac-Beh protein hydrolysates, employing Alcalase® in a 90 min time was 72.60 %.

Evaluation of antioxidant activity with DPPH and ABTS in hydrolysates

The results of the antioxidant activity of the Sac-Beh maize protein hydrolysates by DPPH method show that

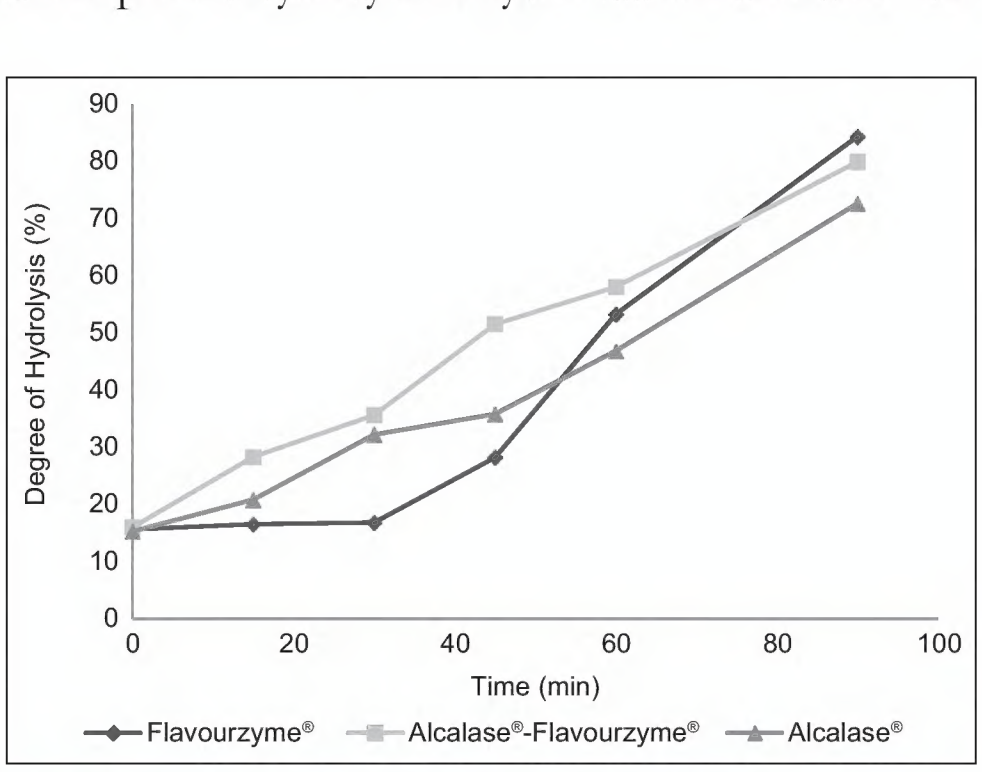

Fig 2. Degree of hydrolysis of Sac-Beh maize (QPM) protein hydrolysate using Alcalase® enzyme, Flavourzyme®, and the Alcalase®-Flavourzyme® system. The results are the average of three replicates

they are dependent on the enzymatic hydrolysis treatment employed, finding statistically significant differences ($P<0.05$) between the hydrolysates themselves and against the ascorbic acid control, whose values are shown in Fig. 3 and had an inhibition of 98.31% that was statistically higher ($P < 0.05$) than the other treatments. The hydrolysate with the highest inhibition was the one with Alcalase® enzyme with a value of 45.51%; this was better compared to Flavourzyme® with 21.12% and 33.35% for the sequential system Alcalase®-Flavourzyme®, all of them at a time of 90 min. The antioxidant activity results obtained in the present study using Alcalase® as an individual system, the sequential system Alcalase®-Flavourzyme® and Flavourzyme® were lower than those obtained by another author in canola protein hydrolysates, at 70.2 %, 69.1 %, and 73.2 % respectively (Cumby et al., 2008). The antioxidant activity in the present study was higher than that in protein concentrates in beans of three varieties with Alcalase® (44.05 %), Thermolysin (34.03 %), and Pancreatin (33.90 %) (Valdez et al., 2012). It was also higher than the 29% reported for soy protein hydrolysates obtained with Alcalase® at a concentration of 100 mg/ml-1 (Zhang et al. 2010), as well as for a protein hydrolysate in African yam hydrolyzed with Alcalase® with 2.29% (Girgih et al. 2015). Likewise, it resulted lower compared to chia (*Salvia hispanica* L.) hydrolysates obtained with Alcalase® (71.11±1.65 %) at 60 min, with Flavourzyme® enzyme at 67.99% at 90 min, and for the sequential system with 77.47±0.50 % also at 90 min (Lopez-Garcia et al., 2019). The specificity of the Flavourzyme® enzyme to release peptides with hydrophobic C-terminal amino acids could explain the increased antiradical activity against DPPH.

Antioxidant activity by ABTS was statistically equal ($P > 0.05$) for all treatments, with values close to 45% but not when compared to BHA (Fig. 4). The Trolox equivalent values of the protein hydrolysates for this case were 8.96 ±0.26 µmol/mg for Flavourzyme®, 9.1 ±0.24 µmol/mg for the Alcalase®- Flavourzyme® system, and 8.98 ±0.24 µmol/mg protein for Alcalase®. This antioxidant capacity is related to the existence of several amino acids such as tyrosine, tryptophan, methionine, lysine, histidine, and cysteine; however, the presence of these amino acids alone may not be sufficient to show the activity, but they also influence the amino acid sequence (Saiga et al., 2003). Research has been conducted on the antioxidant capacity by ABTS in vegetables, for example, spinach with 8.49, cucumbers with 0.43, and pumpkin with 3.74, among others, expressed as Trolox equivalents (TEAC) (Pellegrini et al., 2003). Likewise, with the Flavourzyme® enzyme in sunflower seed, hydrolysate values of 222 µmol Trolox/g were obtained (Esteve et al., 2015). In other studies with Alcalase® 2.4 L, an activity of 460 µmol ET/g was reported for date palm seed hydrolysates (Ambigaipalan et al., 2015). The free radical scavenging ability of protein hydrolysates is explained through several mechanisms: the ability to hydrogen donation, stabilize radicals, scavenge prooxidative metal ions, and likely the ability to form a physical barrier around fat droplets using a particular amino acid (Seo et al., 2015). The values found in this research for QPM hydrolysates constitute an important advance in this field given that there are no works reported in the published literature on the antioxidant capacity of the proteins of this very particular variety of *Zea mays* L.

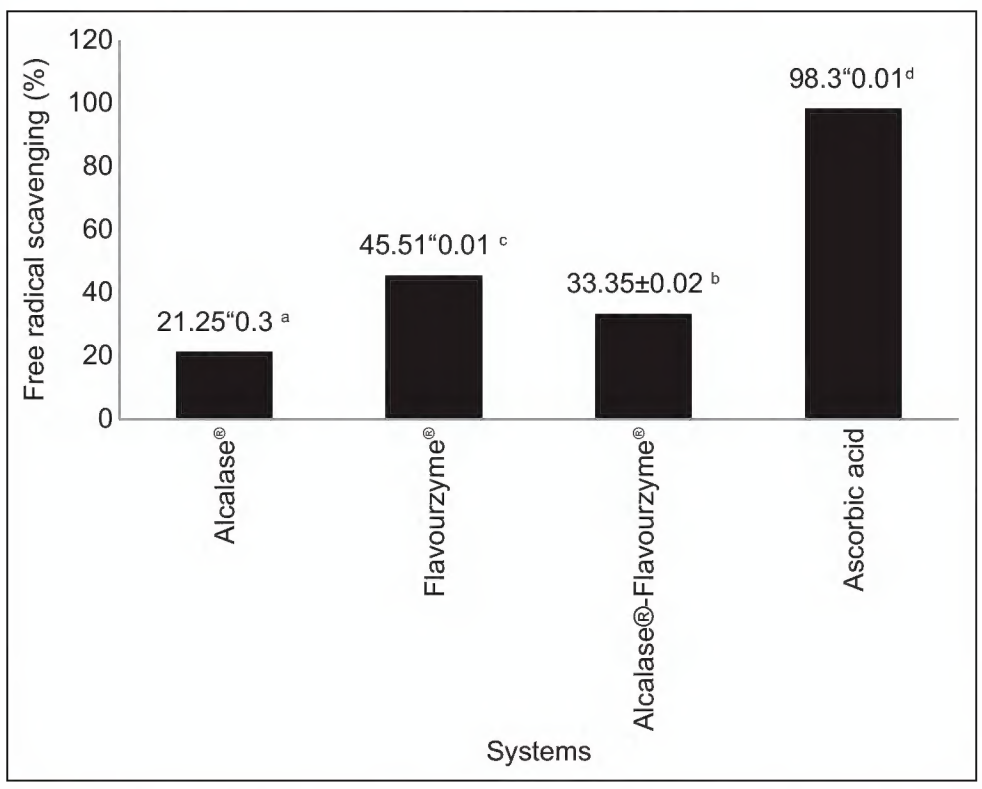

Fig 3. Antioxidant activity of Sac-Beh quality protein maize (QPM) protein hydrolysates by DPPH. [a-d]Different letters indicate that there are statistically significant differences ($P < 0.05$). The results are the average of three replicates.

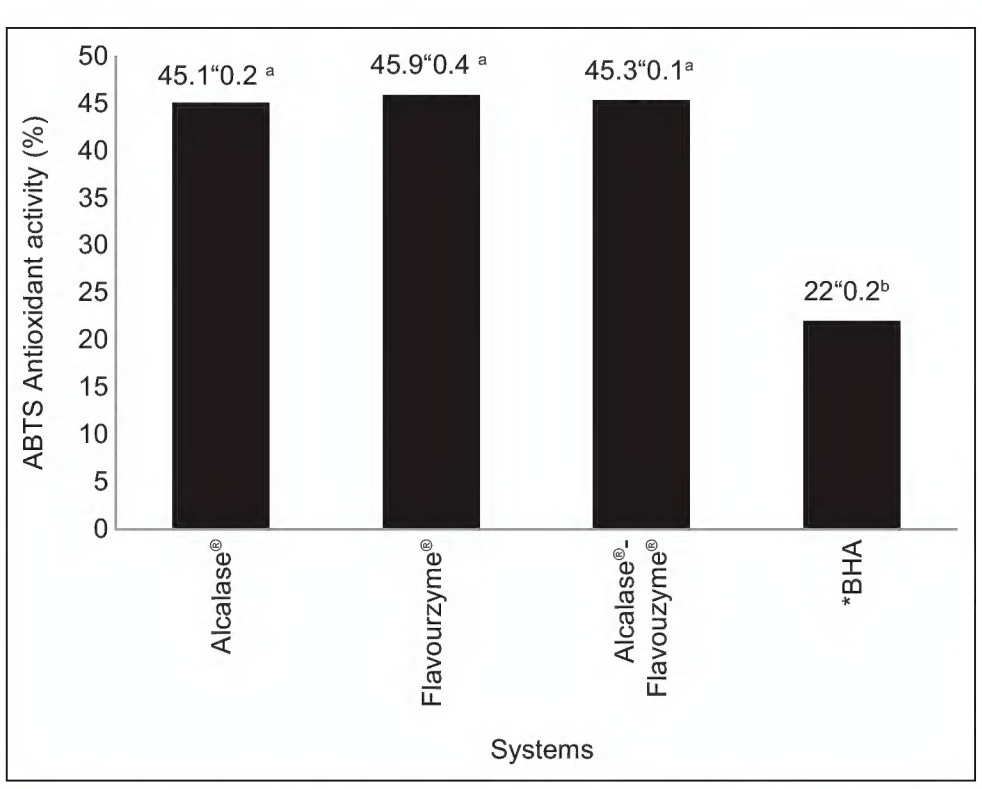

Fig 4. Antioxidant activity of Sac-Beh quality protein maize (QPM) protein hydrolysates by ABTS. [a-b]Different letters indicate that there are statistically significant differences ($P < 0.05$). The results are the average of three replicates. *Vargas-Aispuro et al., 1998.

In vitro inhibition of α-Amylase and α-Glucosidase

The enzyme α-Amylase is responsible for starch hydrolysis during the digestive process, which is important for postprandial management of blood glucose levels. *In vitro* inhibition of Sac-Beh maize hydrolysates on α-Amylase enzyme can be observed in Fig. 5. The results show that there is a significant difference ($p < 0.05$) between the enzymes Alcalase®, Flavourzyme®, and the Alcalase®-Flavourzyme® system. These values were compared with acarbose as a reference inhibitor. The highest inhibition presented was (45.84%) with Alcalase®; this inhibition was approximately 46% of that obtained with acarbose (98.7%) used as a positive control, at a concentration of 50 mg/ml. The lowest inhibition (28.1%) was presented by the Alcalase®-Flavourzyme® system and the Flavourzyme® hydrolysate was in the middle with 32.97%.

In the α-Amylase inhibition of 28.17% presented by the hydrolysate with Alcalase® at a concentration of 107µg/ml, it's important to note that the inhibition is a function of the concentration used of the hydrolysate as reported with the inhibitory activity of *Salvia hispanica* biopeptides at a concentration of 2000 µg (Sosa-Crespo et al., 2018). Although the concentrations evaluated do not produce an inhibition beyond 45.84%, it is possible that increasing their concentration will improve the inhibitory effect on α-Amylase and these could be useful for innovative approaches in the search for new anti-diabetic functional foods based on QPM *Zea mays* L., with less toxicity and as a natural alternative to drugs that cause side effects.

In the case of α-Glucosidase, the results showed that there is a significant difference ($p< 0.05$) between the protein hydrolysates obtained with the enzymes Alcalase®, Flavourzyme®, and the Alcalase®-Flavourzyme® system. These values were compared with acarbose as a reference inhibitor. The highest inhibition was presented by the hydrolysate with the Flavourzyme® enzyme being 64.36%, however, this was lower compared to the percentage obtained by acarbose 98.8% at a concentration of 2 mg/ml. The lowest inhibition (15.7%) was presented by the hydrolysate with Alcalase® with the enzyme α-Glucosidase (Fig. 6). Other studies indicate that walnut (*Juglans mandshurica*) hydrolysates showed inhibition in *in vitro* studies of 61.73 % and 39.08% for both α-Glucosidase and α-Amylase with Alcalase® and Flavourzyme® enzymes respectively (Wang et al., 2020), in basil seeds 36% with Alcalase® (Nurul et al., 2016) and amaranth with Alcalase® (Vilcacundo et al., 2019).

The concentration evaluated in the hydrolysates with the Flavourzyme® enzyme produced an inhibition greater than 64.36% according to the inhibition results obtained on α-Glucosidase, these will be useful for innovative approaches in the search for natural anti-diabetic sources based on QPM *Zea mays* L. since, delaying glucose absorption through inhibition of α-Glucosidase and α-Amylase enzymes, is one of the therapeutic approaches in the management of type 2 diabetes that can reduce the incidence of postprandial hyperglycemia (Tafesse et al., 2020).

Angiotensin-I converting enzyme inhibitory activity

ACE-I inhibitors have been widely used for the prevention and treatment of hypertension (Ko *et al.*, 2012, and Lui *et al.*, 2018). Captopril, a structurally modified synthetic ACE-I inhibitor, strongly inhibits ACE-I, in this case with a value of 98.9% (Fig. 7); in this work, the Alcalase®-Flavourzyme® system was the highest ACE-I inhibitor

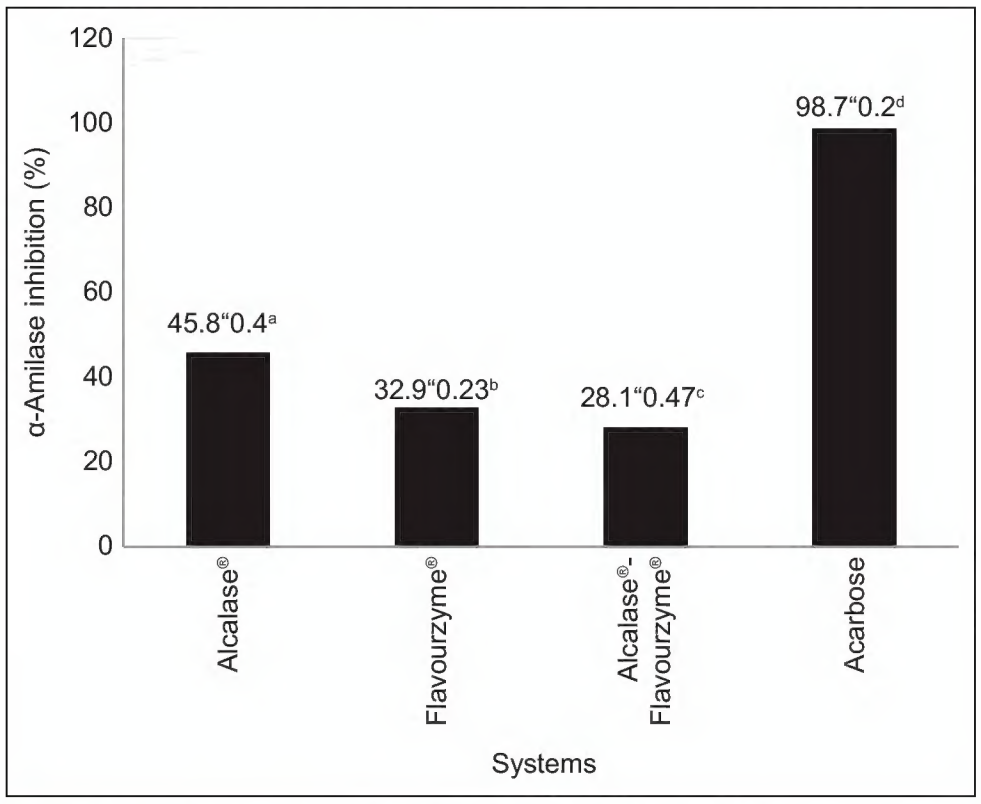

Fig 5. Antihyperglycemic activity of the α-Amylase enzyme on protein hydrolysates of Sac-Beh quality protein maize (QPM). [a-d]Different letters indicate statistically significant differences ($P < 0.05$). The results are the average of three replicates. Control+ acarbose 50mM.

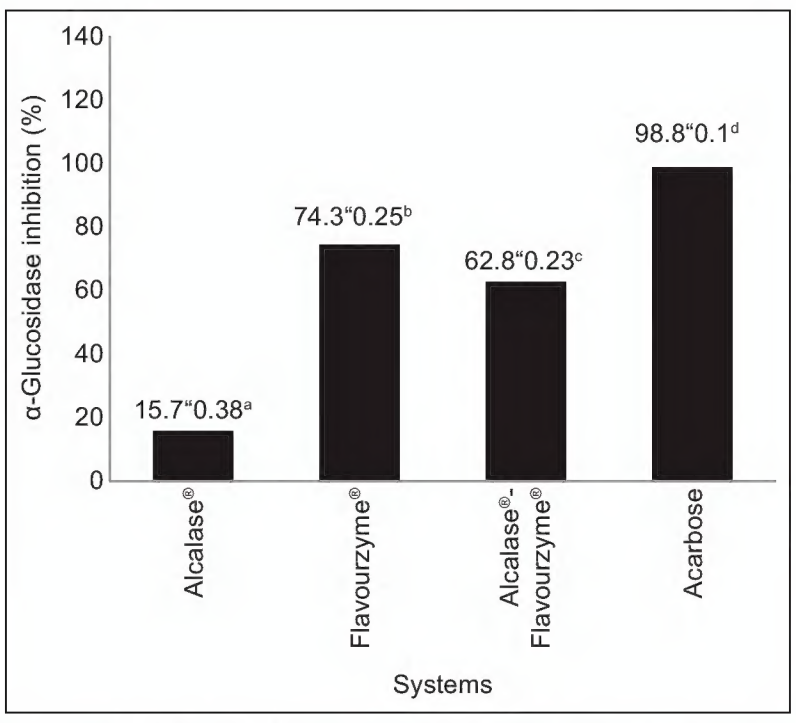

Fig 6. Inhibition (%) of α-Glucosidase enzyme on protein hydrolysates obtained from Sac-Beh quality protein maize (QPM). [a-d]Different letters indicate statistically significant differences ($P < 0.05$). The results are the average of three replicates. Control+ acarbose 50mM.

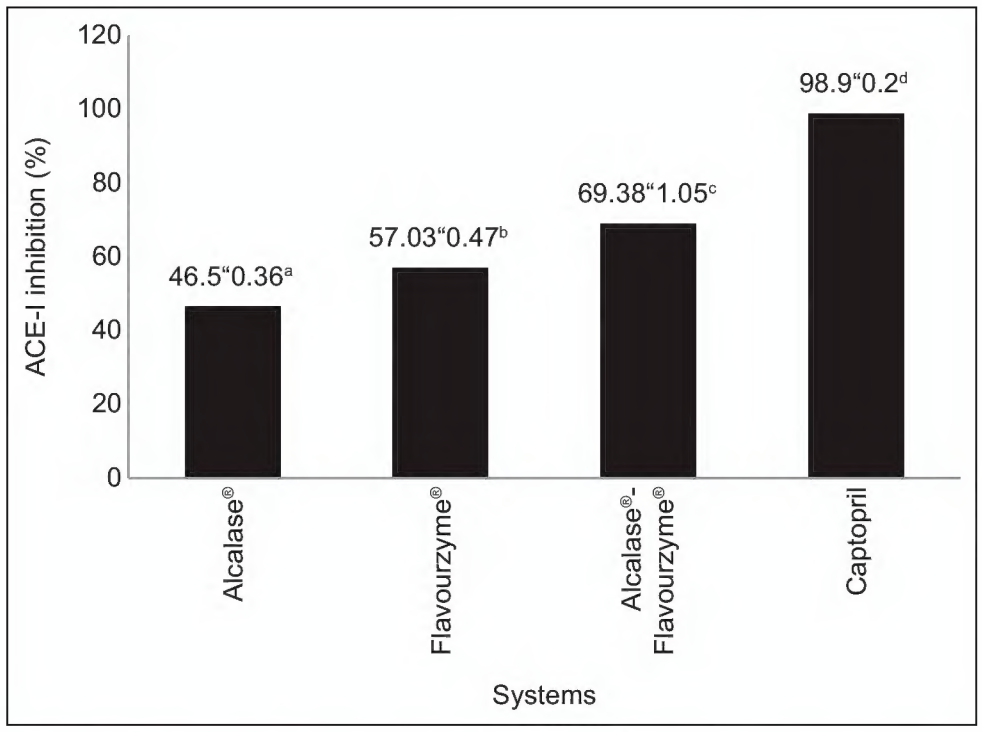

Fig 7. Angiotensin-I enzyme inhibitory activity percentage on protein hydrolysates obtained from quality protein Sac-Beh maize (QPM). [a-d]Different letters indicate statistically significant differences ($P < 0.05$). Control+ =Captopril.

with a value of 69.067 %, however, it is still lower than the inhibition presented by Captopril but higher ($P<0.05$) compared to the inhibition of Flavourzyme® with 57.03% and of Alcalase® with 46.6%. However, it is known that the administration of Captopril causes various side effects such as headaches, insomnia, and fever (Ko et al., 2012). Therefore, the hydrolysates studied here could be used as safe antihypertensive agents without these downsides. Most of the hydrolysates with antihypertensive properties described in the published literature can inhibit the Angiotensin-I converting enzyme (ACE-I), a key regulator in the renin-angiotensin system, which leads to the production of angiotensin II; a peptide that acts as a vasoconstrictor agent involved in the exacerbation of hypertension (Ganguly et al., 2019). However, Alcalase® and Flavourzyme® tend to generate peptides with hydrophobic amino acid C-terminus. It has been demonstrated by molecular methodologies that peptides containing hydrophobic amino acids at their C-terminus show potentially strong ACE-I inhibition. Therefore, Alcalase® and Flavourzyme® enzymes are likely to be suitable for the preparation of highly active ACE-I inhibitor hydrolysates by increasing the concentration to be used. Additionally, these two proteases are easily obtained at a relatively low cost because they are microbial enzymes and are recommended for industrial use (Segura-Campos et al., 2013).

As an indicator of the concentration of hydrolysates activity required to produce a 50% inhibition of ACE-I, (IC_{50}) was used, and the Alcalase®-Flavourzyme® system was 73.09µg/ml. This result is a low value indicating that Sac-Beh QPM hydrolysates have an inhibitory power on ACE-I. However, the value recorded in this research is higher than those reported for different protein sources. In this regard, Segura-Campos et al., (2013) hydrolyzed the protein-rich fraction of chia (*Salvia hispanica* L.) with the Alcalase®-Flavourzyme® sequential system during 90, 120, and 150 min of digestion, obtaining IC_{50} values of 44.01, 20.76 and 8.86 µg/ml, respectively. The IC_{50} value (73.09 µg protein/ml) for the hydrolysates of the QPM isolates found in the present study has been compared with the inhibitions of the drugs Captopril and Enalapril (IC_{50} = 0.021 and 0.00105 µg/ml) currently used in clinical treatments in a study performed in rats with spontaneous hypertension and in patients suffering from arterial hypertension (Cushman et al., 1989). This indicates that the QPM hydrolysates have lower activity, but with the potential to be considered ACE-I inhibitors. The hydrolysates of the QPM isolate constitute a good source for obtaining bioactive peptides with ACE-I activity and could be used in the incorporation of food systems for the elaboration of functional foods and, due to their potential inhibitory effect on ACE-I, could be applied in the future in treatments for people with hypertension.

CONCLUSIONS

The protein hydrolysates obtained with different enzymatic systems from Sac-Beh maize are classified as extensive since they had a value higher than 10% degree of hydrolysis. This study demonstrates that Sac-Beh *Zea mays* L. QPM protein hydrolysates, obtained with Flavourzyme®, Alcalase® enzymes, and the Alcalase®-Flavourzyme® system under certain conditions of time, temperature, and pH constitute a source of raw material for obtaining protein hydrolysates with bioactive potential. It can be considered that the protein hydrolysate of *Zea mays* L. QPM has peptide fractions that act as inhibitors of ACE-I, α-Amylase, and α-Glucosidase, and also as antioxidants, desirable for use and incorporation in systems for the elaboration of functional foods; they also could be applied in future treatments for people with hypertension and diabetes.

ACKNOWLEDGMENTS

This research was supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT-México), through doctoral grant number 417656.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

Authors contributions

Nidia Jímenez Suaste is the principal autor and participed in the acquisition and analysis of data, and writing the

paper. Luis Chel Guerrero participated in the adaptation of some analytical techniques and the acquisition of data. Roberto Zamora Bustillos participated in the design of the research, analysis of data and was involved in revising the paper critically. David Betancur Anocna participated in the design of the research, analysis of data and revising the paper critically.

REFERENCES

Aldhanhani, A. R., Z. F. Ahmed, N. Tzortzakis and Z. Singh. 2022a. Maturity stage at harvest influences antioxidant phytochemicals and antibacterial activity of jujube fruit (*Ziziphus mauritiana* Lamk. and *Ziziphus spina-christi* L.). Ann. Agric. Sci. 67: 196-203.

Aldhanhani, A. R. H., N. Kaur and Z. F. R Ahmed. 2022b. Antioxidant phytochemicals and antibacterial activities of sidr (*Ziziphus* spp.) leaf extracts. Acta Hortic. 1353: 323-332.

Al Shaibani, F. Y. Y., N. Kaur and Z. F. R. Ahmed. 2022. Reducing postharvest loss by improving fruit quality, shelf life, bioactive compounds of Rutab date (*Phoenix dactylifera* L. 'Barhi') using natural elicitors. Acta Hortic. 1340: 119-124.

AOAC. 2012. In: W. Horwitz (Ed.), Official Methods of Analysis. 19th ed. Association of Official Analytical Chemists, Washington, DC.

Ambigaipalan, P., A. S. Al-Khalifa and F. Shahidi. 2015. Antioxidant and angiotensin I using alcalase, flavourzyme and thermolysin. J. Funct. Foods 18: 1125-1137.

Chalamaiah, M., W. Yu and J. Wu. 2018. Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: A review. Food Chem. 245: 205-222.

Chuesiang, P. and R. Sanguandeekul. 2015. Protein hydrolysate from tilapia frame: Antioxidant and angiotensin I converting enzyme inhibitor properties. Int. J. Food Sci. Techol. 50: 1436-1444.

Cumby, N., Y. Zhong, M. Naczk, and F. Shahidi. 2008. Antioxidant activity and water-holding capacity of canola protein hydrolysates. Food Chem. 109: 144-148.

Cushman, D., F. Wang, W. Fung, G. Grover, C. Harvey, R. Scalese, S. L. Mitch and J. M. De Forrest. 1989. Comparisons *in vitro*, *ex vivo*, and *in vivo* of the actions of seven structurally diverse inhibitors of angiotensin converting enzyme (ACE). Br. J. Clin. Pharmacol. 28: 115-131.

De Castro, R. J. S., V. G. Cason and H. H. Sato. 2017. Binary mixture of proteases increases the antioxidant properties of white bean (*Phaseolus vulgaris* L.) protein-derived peptides obtained by enzymatic hydrolysis. Biocat. Agric. Biotechnol. 10: 291-297.

Du-Xin J., L. Xiao-Lan, Z. Xi-Qun, W. Xiao-Jie and H. Jun-Fang. 2016. Preparation of antioxidative corn protein hydrolysates, purification and evaluation of three novel corn antioxidant peptides. Food Chem. 204: 427-436.

Esfandi, R., W. G. Willmore and A. Tsopmo. 2019. Peptidomic analysis of hydrolyzed oat bran proteins, and their *in vitro* antioxidant and metal chelating properties. Food Chem. 279: 49-57.

Esteve, C., M. L. Marina and M. C. García. 2015. Novel strategy for the revalorization of olive (*Olea europaea*) residues based on the extraction of bioactive peptides. Food Chem. 167: 272-280.

Fitzgerald, C., E. Gallagher, L. Doran, M. Auty, J. Prieto and M. Hayes. 2014. Increasing the health benefits of bread: Assessment of the physical and sensory qualities of bread formulated using a renin inhibitory *Palmaria palmata* protein hydrolysate, LWT. Food Sci. Technol. 56: 398-405.

Ganguly, A., K. Sharma and K. Majumder. 2019. Chapter Four-Food-derived bioactive peptides and their role in ameliorating hypertension and associated cardiovascular diseases. Adv. Food Nutr. Res. 89: 165-207.

Girgih, A. T., R. He, F. M. Hasan, C. C. Udenigwe, T. A. Gill and R. E. Aluko. 2015. Evaluation of the *in vitro* antioxidant properties of a cod (*Gadus morhua*) protein hydrolysate and peptide fractions. Food Chem. 173: 652-659.

Hasjim, J., S. Srichuwong, M. P. Scott and J. L. Jane. 2009. Kernel composition, starch structure, and enzyme digestibility of opaque-2 maize and quality protein maize. J. Agric. Food Chem. 57: 2049-2055.

Hayakari, M., Y. Kondo and H. Izumi. 1978. A Rapid and simple espectrophotometric assay of angiotensin-converting enzyme. Anal. Biochem. 84: 361-369.

Karimi, A., H. A. Gavlighi, R. A. Sarteshnizi and C. C. Udenigwe. 2021. Effect of maize germ protein hydrolysate addition on digestion, *in vitro* antioxidant activity and quality characteristics of bread. J. Cereal Sci. 97: 103148.

Ko, S. C., N. Kang, E. A. Kim, M. C. Kang, S. H. Lee, S. M. Kang, J. B. Lee, B. T. Jeon, S. K. Kim, S. J. Park, P. J. Park, W. K. Jung, D. Kim and Y. J. Jeon. 2012. A novel angiotensin I-converting enzyme (ACE) inhibitory peptide from a marine *Chlorella ellipsoidea* and its antihypertensive effect in spontaneously hypertensive rats. Process Biochem. 47: 2005-2011.

Kulczyk, A., M. Karamac and A. Kosińska-Cagnazzo. 2016. Use of different proteases to obtain flaxseed protein hydrolysates with antioxidant activity. Int. J. Mol. Sci. 17: 421-429.

López-García, S. L., G. Arambula-Villa, J. G. Torruco-Uco, A. C. Oliva, F. H. Rosas, M. López-Espíndola and J. A. Corredor. 2019. Actividad antioxidante de la fracción proteica de chía (*Salvia hispanica* L.) hidrolizada con alcalasa y flavourzima. Agrociencia, 53: 505-520.

Lowry, O., N. Rosebrough, A. Farr and R. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Margot, A., E. Flaschel and A. Renken. 1997. Empirical kinetic models for tryptic whey-protein hydrolysis. Process Biochem. 32: 217-223.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428.

Mojica, L. and E. González de Mejía. 2015. Characterization and comparison of protein and peptide profiles and their biological activities of improved common bean cultivars (*Phaseolus vulgaris* L.) from Mexico and Brazil. Plant Foods Hum. Nut. 70: 105-112.

Nurul Hidayatul Afifah, B. S. S. and C. Y. Gan. 2016. Antioxidative and amylase inhibitor peptides from Basil seeds. Int. J. Pept. Res. 22: 3-10.

Pasarin, D. and C. Rovinaru. 2023. Chemical analysis and protein enzymatic hydrolysis of poly-floral bee pollen. Emir. J. Food Agric. 35: 59-69.

Pellegrini, N., M. Serafini, B. Colombi, D. Del Rio, S. Salvatore, M. Bianchi and F. Brighenti. 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. J. Nutr. 133: 2812-2819.

Pukalskas, A., T. A. van Beek, R. P. Venskutonis, J. P. Linssen, A. van Veldhuizen and A. E. de Groot. 2002. Identification of radical scavengers in sweet grass (*Hierochloe odorata*). J. Agric. Food Chem. 50: 2914-2919.

Saiga, A., S. Tanabe and T. Nishimura. 2003. Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease

treatment. J. Agric. Food Chem. 51: 3661-3667.

Segura-Campos, M. R., I. M. Salazar-Vega, L. A Chel-Guerrero and D. A. Betancur-Ancona. 2013. Biological potential of chia (*Salvia hispanica* L.) protein hydrolysates and their incorporation into functional foods. LWT. Food Sci. Technol. 50: 723-731.

Seo, H. W., E. J. Jung, G.W. Go, G. D. Kim, S. T. Joo and H. S. Yang. 2015. Optimization of hydrolysis conditions for bovine plasma protein using response surface methodology. Food Chem. 185: 106-111.

Shahidi, F. and P. Ambigaipalan. 2015. Novel functional food ingredients from marine sources. Curr. Opin. Food Sci. 2: 123-129.

Shimada, K., K. Fujikawa, K. Yahara and T. Nakamura. 1992. Antioxidative properties of xanthan on the auto oxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40: 945-948.

Sosa-Crespo, I., H. Laviada-Molina, L. Chel-Guerrero, R. Ortiz-Andrade and D. Betancur-Ancona. 2018. Efecto inhibitorio de fracciones peptídicas derivadas de la hidrólisis de semillas de chía (*Salvia hispanica*) sobre las enzimas α-amilasa y α-glucosidasa. Nut. Hosp. 35: 928-935.

Tafesse, T. B., M. H. Bule, M. Khoobi, M. A. Faramarzi, M. Abdollahi and M. Amini. 2020. Coumarin-based scaffold as α-glucosidase inhibitory activity: Implication for the development of potent antidiabetic agents. Mini Rev. Med. Chem. 20: 134-151.

Valdez-Ortíz, A., C. I. Fuentes-Gutiérrez, L. J. Germán-Báez, R. Gutiérrez-Dorado and S. Medina-Godoy. 2012. Protein hydrolysates obtained from Azufrado (Sulphur yellow) beans (*Phaseolus vulgaris*): Nutritional, ACE-inhibitory and antioxidative characterization. LWT. Food Sci. Technol. 46: 91-96.

Vargas-Arispuro, L., B. I. Sanz, M. A. Martínez-Téllez and E. Primo-Yúfera. 1998. Actividad antioxidante de compuestos aislados en residuos no volátiles de aceite esencial de naranja. Grasas Aceites. 49: 159-164.

Vasal, S. K, G. Srinivasan, S. Pandey, C. F. González, J. Crossa and D. L. Beck. 1993. Heterosis and combining ability of CIMMYT's quality protein maize germplasm. Crop Sci. 33: 46-51.

Vilcacundo, R., C. Martínez-Villaluenga, B. Miralles and B. Hernández-Ledesma. 2019. Release of multifunctional peptides from kiwicha (*Amaranthus caudatus*) protein under *in vitro* gastrointestinal digestión. J. Sci. Food Agric. 99: 1225-1232.

Wang, C. and L. A. Johnson. 2001. Functional properties of hydrothermally cooked soy protein products. J. Am. Oil Chem. Soc. 78: 189-195.

Wang, J., T. Wu, L. Colmillo, C. Liu, X. Liu, H. Li, J. Shi, M. Li and W. Min. 2020. Anti-diabetic effect by walnut (*Juglans mandshurica* Maxim.) derived peptide LPLLR through inhibiting α-glucosidase and α-amylase, and alleviating insulin resistance of hepatic HepG2 cells. J. Funct. Foods. 69: 103944.

Zhang, L., J. Li and K. Zhou. 2010. Chelating and radical scavenging activities of soy protein hydrolysates prepared from microbial proteases and their effect on meat lipid peroxidation. Bioresour. Technol. 101: 2084-2089.